

## Claims

[001] An “induction solution” which provides for rapid detection of coliforms, capable to induce, in absence of cell growth, the expression of inducible enzymes  $\beta$ -glucuronidase and  $\beta$ -galactosidase, comprising:

- at least one amino acid, yet preferably a mixture of amino acids, in such a quantity not to allow, between 0 and 120 minutes, a detectable cell growth of the coliforms in contact with it;
- a buffer system;
- bivalent ions and more particularly, the magnesium  $Mg^{++}$ , in a concentration preferably 0.5 mM;
- an enzymatic inducer.

[002] A solution according to claim 1 wherein the amino acid concentration reaches up to about 80mM.

[003] A solution according to claims 1-2 comprising at least one of the following amino acids:

- tryptophan W;
- at least one between methionine M and threonine T;
- isoleucine I and leucine L.

[004] A solution according to claim 1 wherein the amino acid is selected from the group of natural amino acids: alanine A, cysteine C, aspartic acid D, glutamic acid E, phenylalanine F, glycine G, histidine H, isoleucine I, lysine K, leucine L, methionine M, asparagine N, proline P, glutamine Q, arginine R, serine S, threonine T, valine V, tryptophan W, tyrosine Y.

[005] A solution according to claims 1-4 wherein the mixture of amino acids comprises the natural amino acids in levorotatory form, ranging from about 0.01 to about 0.05 mM each.

[006] A solution according to claims 1-5 wherein the buffer system is a phosphate buffer providing a pH between 6.0 and 7.5 and comprising sodium chloride (NaCl), preferably, but not necessarily, at about 0.01% (w/v).

[007] A solution according to claims 1-6 wherein the enzymatic inducer is selected among isopropyl- $\beta$ -D-thiogalactopyranoside for  $\beta$ -galactosidase, concentration being preferably about 0.2mM, methyl- $\beta$ -D-glucuronide for  $\beta$ -glucuronidase, concentration being preferably about 2mM, and relative mixtures.

[008] A solution according to claims 1-7 utterly comprising a selective agent acting as a membrane permeabilizer like, for instance, sodium dodecyl sulphate.

[009] An analysis kit for the rapid detection of coliform cells comprising:

- the induction solution A according to claims 1-8;

- a solution comprising a fluorescent substrate;
- instructions for use;
- suitable solvents and instrumentation.

[010] A kit according to claim 9 wherein the coliforms are selected among total coliforms, faecal coliforms, *E. coli*.

[011] A kit according to claim 9 wherein the induction solution is filter-sterilized by passage through 0.45 µm or less pore size membrane filter and is in a lyophilized form.

[012] A kit according to claim 9 wherein the fluorescent substrate is selected from the group consisting of methylumbelliferyl-β-D-galactoside, methylumbelliferyl-β-D-glucuronide and relative mixtures.

[013] A kit according to claim 9 utterly comprising a fluorescence amplifier constituted from a solution of sodium hydroxide (NaOH) in water or any other base capable to increase pH of the induction solution to about 11-12.

[014] A kit according to claim 9 utterly comprising an organic solvent, for example chloroform or any other agent capable to induce cell lysis, for example triton-x.

[015] A kit according to claims 9-14 for the analysis of water samples, comprising wastewater, surface water, bathing water, freshwater, seawater, groundwater, food extracts and soil extracts.

[016] A process for the rapid detection of coliform bacteria, comprising the steps of:  
- placing the sample to be analyzed in contact with an induction solution A, according to claims 1-8 and incubating between 30 and 50°C;  
- adding a fluorogenic substrate;  
- rupturing the cells with any suitable lysis agent to release the induced enzyme;  
- assisting hydrolysis of fluorogenic substrate with discharge of the corresponding fluorophore by incubating at less than 50°C, for a period of time depending on cell number;  
- raising final mixture pH to about 11-12 with a basic solution, for example sodium hydroxide (NaOH) in water.

[017] A process according to claim 16 wherein the temperature is between 30 and 40°C for the detection of total coliforms and between 30 and 50°C for the detection of faecal coliforms or *E. coli*.

[018] A process according to claim 16 wherein a minimum time period of about 10 minutes is required for the enzymatic reaction.

[019] Process according to claim 16 wherein the fluorophore concentration is determined by fluorimetric assay after extracellular hydrolysis reaction of target enzymes with relative substrates being, respectively, methylumbelliferyl-β-D-galactoside for β-galactosidase and methylumbelliferyl-β-D-glucuronide for

β-glucuronidase.

[020] Process according to claim 16 wherein the lysis agent is chloroform and/or triton-x.

[021] Process according to claim 16 wherein the fluorescent substrate is selected from the group comprising methylumbelliferyl-β-D-galactoside in dimethylsulfoxide, methylumbelliferyl-β-D-glucuronide in water/triton-X and relative mixtures.

[022] Process according to claim 16 wherein the sample to be analyzed is pre-extracted with physiological saline or phosphate buffered saline solution.

[023] Process according to claim 22 wherein the sample is utterly filtered on a 0.45 µm or less membrane filter and the filter is immersed in the induction solution.

[024] Process according to claim 16 wherein the sample analysis is performed by fluorimetry, being the excitation wavelength set between 330 and 390 nm and the emission wavelength set between 410 and 470 nm with slit widths between 2.5 and 20 nm.